Clinical Implications

- Signal transducer and activator of transcription 3 (STAT3) activity and paired box 3 (PAX3) levels may modulate responses to RAF inhibitors in mutant BRAF melanomas.
- STAT3 inhibitors are currently being tested in clinical trials.
- Both autocrine and paracrine mechanisms may regulate STAT3–PAX3 signaling in response to RAF inhibitors.

examination of WP1066. Earlier work with WP1066 has demonstrated its ability to block phosphorylation of JAK2 and STAT3, reduce melanoma proliferation, and diminish tumor growth *in vivo* (Kong *et al.*, 2008). In this study, WP1066 inhibited phosphorylation of STAT3 and reduced downstream levels of PAX3, irrespective of vemurafenib sensitivity status. In addition, combined treatment with vemurafenib and WP1066 decreased the number of vemurafenib-resistant cells more effectively than either drug alone. Although this work has yet to determine whether there is mechanistic cooperation between V600E BRAF inhibition through vemurafenib- and WP1066-elicited reduction in activated STAT3, it suggests that STAT3 targeting in melanoma may be effective. Dosing curves of these drugs in conjunction with either knockdown or overexpression studies may provide better insight into potential synergies. Because STAT3 signaling seems to be a necessary pathway for melanoma cell viability, these findings have translational implications as they may provide a broad therapeutic strategy for targeting the heterogeneity of vemurafenib-resistance mechanisms, akin to the notion recently proposed for HSP90 inhibitors (Paraiso *et al.*, 2012). Although STAT3 inhibitors such as WP1066 have yet to be evaluated fully in the clinic, JAK2/STAT3 inhibitors are currently in phase I/II clinical trials for head and neck tumors and lymphomas. This study lays a foundation for additional preclinical studies on the use of WP1066 and other STAT3 inhibitors in patients with vemurafenib-resistant melanomas.

CONFLICT OF INTEREST
The authors state no conflict of interest.

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See related article on pg 2065

Clinical Implications of CD8+ T-Cell Infiltration in Frequent and Rare Cancers

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Recent studies of cancer patients revealed high diversity in oncogenic mechanisms, leading to increased treatment individualization for subgroups of patients with frequent cancers. A similar development may not be possible for patients with rare cancers, such as Merkel cell carcinoma (MCC). Finding shared disease mechanisms may open new options to understanding and treating such tumors. Tumor-infiltrating CD8+ T cells are frequently associated with favorable clinical outcome in a remarkably large spectrum of cancers. In this issue, Afanasiev *et al.* suggest a mechanism that may hinder the tumor homing of CD8+ T cells in MCC patients. It is possible that therapeutic mobilization of anti-cancer T cells may be useful in patients who share this specific immune biological feature.

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The tumor microenvironment

Cancer cells are surrounded by stromal cells, blood and lymphatic vessels, and immune cells. The tumor microenvironment (TME) is not only shaped primarily by the tumor itself but also strongly influenced by the host. Our understanding of the role of the immune system in the TME has increased steadily in the last decade. Although inflammatory

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Clinical Implications

- Human cancers can present both common and individual biological features.
- Cellular and molecular profiling by immunohistochemistry and molecular biology techniques are promising approaches in identifying the characteristics of individual cancers.
- Profiling of individual cancers not only allows better prognosis evaluation but may also open new targeted treatment options.

mechanisms often promote tumor growth (Hanahan and Weinberg, 2011), the immune response has the potential for tumor control, particularly through activated CD8+ T cells (Fridman *et al.*, 2012). Characterization of the interplay between factors that support and factors that inhibit anti-cancer T cells is complicated by the fact that immune infiltrates are: (1) heterogeneous, (2) vary among tumor types, and (3) differ from patient to patient.

Immune signatures, lymphocytic infiltration, and roles of lymphocytes in human cancer

Various immune cell types may be found in the core of tumors, in the invasive front as well as in the adjacent stroma and lymphoid structures. Lymphocytes are located in specific areas, whereas natural killer cells are found in the stroma, without direct contact with the tumor cells. B cells are generally found in the invasive margins of growing tumors, and T cells, particularly cytotoxic CD8+ T cells, may be located in the invasive margins and in the tumor core. Of major interest, enhanced intratumoral CD8+ T-cell infiltration has been reported to correlate with good clinical outcome in the majority of cancers, such as melanoma, head and neck, breast, bladder, urothelial, ovarian, colorectal, prostatic, and lung cancers. An exception to this ''rule'' was found in renal cancer; one study reported a similar correlation, whereas another yielded the opposite result. The role of regulatory CD4+ T cells (Tregs) seems to be more complex, with infiltration correlating with good prognosis in head and neck cancer, bladder cancer, and Hodgkin's lymphoma, and poor prognosis in ovarian, breast, and hepatocellular carcinoma (Fridman *et al.*, 2012). Discrepancies may be explained in part by the existence of different Treg subpopulations and distinct tumor microenvironments (Conrad *et al.*, 2012). Moreover, the role of each lymphocyte population cannot be interpreted in an isolated manner, as the balance between cytotoxic and regulatory lymphocytes is fundamental.

Recently, an immune score has been proposed, based on the enumeration of CD45RO+ memory cells and CD8+ cytotoxic memory cells in the core and in the invasive margin of a patient's tumor, correlating with clinical outcome. Immunohistochemical immune scoring is currently being evaluated in an innovative multicenter study of colon cancer patients (Galon *et al.*, 2012). It is reasonable to postulate that a score based on the comprehensive inclusion of many different immune cell types would correlate even more accurately with the prognosis of individual cancers, and it may further help in defining prognosis and therapy outcome predictions.

Merkel cell carcinoma: characteristics shared with frequent cancers

Studies of Merkel cell carcinoma (MCC) are hampered by the paucity of cases. MCC is a highly aggressive skin cancer expressing neuroendocrine markers. Factors strongly associated with the development of MCC include fair skin, a history of extensive sun exposure, chronic immune suppression (organ transplants or HIV), and age over 50 years. The incidence in the United States has increased fourfold in the past 20 years, making MCC the second most common cause of nonmelanoma skin cancer death (Iyer *et al.*, 2011; Cirillo *et al.*, 2012). In 2008, Feng *et al.* (2008) identified the Merkel cell polyomavirus, which integrates its viral DNA in the host genome in 80% of MCC. Recent studies have characterized immune biological parameters of MCC, showing that strong intratumoral CD8+ T-cell infiltration was associated with a better prognosis. Reminiscent of the majority of frequent cancers, intratumoral CD8+ T-cell infiltration detected by immunostaining on paraffin-embedded tissue, together with initial staging, were significant predictive parameters, but not CD8+ T cells localized to the tumor–stroma interface (neither age nor gender; Paulson *et al.*, 2011). In this issue of the *Journal of Investigative Dermatology*, the same group of researchers publishes a continuation of their studies and proposes a possible mechanism of CD8+ T-cell homing into MCC (Afanasiev *et al.*, 2013).

Nitric oxide, E-selectin, and T cells in MCC

The new study by Afanasiev *et al.* (2013) shows that intratumoral vascular E-selectin, a critical molecule for the entry of CLA+ T cells into tumors, is downregulated in the majority of MCCs, offering a plausible explanation for low CD8+ T-cell infiltration. Downregulation of E-selectin was associated with local nitric oxide (NO) production (as shown in other cancer types), which was found to be higher in tumor samples devoid of CD8+ T cells. Thus, the new study provides evidence for possible mechanisms of intratumoral T-cell infiltration and for tumor immune suppression, correlated with clinical outcome in MCC patients.

Homing mechanisms of T cells: novel therapeutic options

The study hints toward the illustrated immune mechanism of evasion (Figure 1, upper panel): In tumors with a good prognosis, relevant cytotoxic CD8+ T cells circulate in blood vessels that supply the tumor, bind to E-selectin and other integrins, allowing CD8+ T-cell attachment, and diapedesis along chemokine gradients toward the tumor, where they are activated and, in turn, induce apoptosis of tumor cells. By

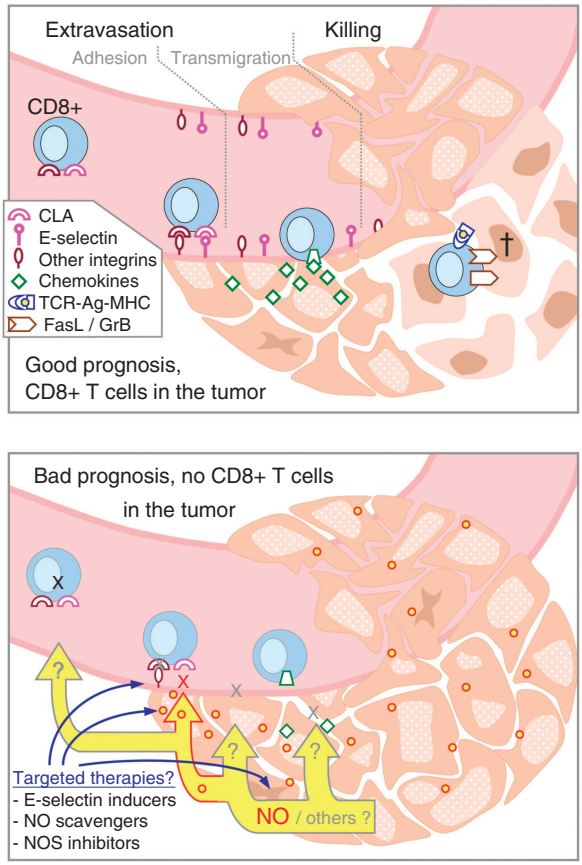

Figure 1. Tumor prognosis. Upper panel: Tumor with a good prognosis. Cytotoxic CD8+ T cells circulate in the blood vessels that supply the tumor. E-selectin and other integrins are upregulated (by chemoattraction), allowing CD8+ T cells to bind to blood vessel walls (first rolling adhesion, then tight adhesion) and to migrate (endothelial transmigration) toward the tumor along a chemokine gradient. Once in the tumor, activated anti-tumor CD8+ T cells induce apoptosis of the tumor cells (through FasL- and granzyme B-mediated killing). Lower panel: In tumors with poor prognosis, high nitric oxide (NO) production inhibits E-selectin expression, blocking CD8+ T-cell extravasation (Afanasiev *et al.*, 2013; red arrow). This model suggests that blocking NO production, lowering NO levels, or directly increasing E-selectin expression may induce CD8+ T-cell entry into the tumor (blue arrows). However, the tumor may block CD8+T-cell entry into the tumor at every other step of extravasation (grey arrows).

contrast, in tumors with a poor prognosis (Figure 1, lower panel), high NO production by the tumor (NO synthetase) inhibits the expression of E-selectin and, possibly, additional homing molecules, hindering entry of CD8+ T cells. The models suggest that blocking NO production, lowering NO levels, or directly increasing integrin and adhesion receptor expression may induce CD8+ T-cell entry into the tumor and subsequent destruction of tumor cells (Figure 1, lower panel). Despite the evidence provided by the study by Afanasiev *et al.* (2013), there is no proof that the proposed mechanism is really responsible for (lack of) T-cell homing. Further studies are required to demonstrate that blocking NO reverses E-selectin levels and that increasing E-selectin is sufficient in itself for enhancing CD8+ T-cell-mediated killing. In fact, although E-selectin is associated with a better prognosis in MCC, the opposite was found in other cancers types, where E-selectin has been reported as a marker of metastatic potential (Afanasiev *et al.*, 2013). Moreover, there are several other nonexclusive ways in which the tumor might avoid immune cell immigration. The tumor might block E-selectin through pathways other than NO, it might act on other integrins required for diapedesis, or block chemokine-mediated T-cell entry, all of which could also explain poor CD8+ T-cell infiltration in MCC. Homing and recruitment of immune cells to tumor sites is also controlled by cytokines and chemokines produced by T cells and other immune cells in the TME (Bos *et al.*, 2012). Multiple actors are triggered by NF-κB activation and by a variety of additional inflammatory factors, some of which have been suggested as prognostic markers of MCC (Fernandez-Figueras *et al.*, 2007). In addition, CD8+ T cells that reach a tumor may not be sufficiently active for appropriate immune-mediated protection. Indeed, intratumoral CD8+ T cells are often hyporesponsive (Baitsch *et al.*, 2012). Finally, the potential role of CD4+ T cells, including Tregs, remains to be characterized.

Despite the remaining questions, current evidence suggests that MCC patients may profit from immunotherapy. Ipilimumab is a mAb, which had been approved in 2011 for the treatment of melanoma, which blocks the inhibitory receptor CTLA-4 on T cells, resulting in enhanced T-cell activation. Additional therapies with comparable modes of action are in development. Reagents blocking PD-1 or PD-L1 are already in clinical trials with promising clinical responses in patients with non-small-cell lung cancer, melanoma, or renal-cell cancer (Ribas, 2012). These and other approaches of immunotherapy can now be promoted to treat MCC patients. In turn, discoveries of disease mechanisms and therapeutic innovations in MCC increases our knowledge, and are also possibly useful in other cancers.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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